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Note

Simultaneous determination of procainamide and N-acetylprocainamide in serum by gas chromatography with nitrogen-selective detection

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Procainamide (PA) is commonly used for the treatment of atrial and ventricular arrhythmias. It has been reported [1] that the therapeutic range is narrow (4–10 μg/ml), the rate of metabolism by hepatic N-acetyltransferase is variable from person to person genetically and the metabolite of PA, N-acetylprocainamide (NAPA), has a similar activity to PA. Therefore, methods for the determination of serum concentrations of both PA and NAPA are required.

The determination of PA in blood by gas chromatography (GC) [2–5], high-performance liquid chromatography (HPLC) [6–12], thin-layer chromatography [13] and enzyme immunoassay [14] has been reported. The reported GC and HPLC methods require relatively complicated extraction procedures, and the GC methods also require a large sample size (more than 1 ml of serum) for routine use. Therefore, we attempted to develop a new method for determining PA and NAPA simultaneously by GC using nitrogen-selective detection. As the nitrogen–phosphorus detector has a high selectivity, it provides an effective means of simplifying the extraction procedure. The simultaneous determination of PA and NAPA by using a nitrogen–phosphorus detector, however, has not previously been reported.

In this work, the extraction procedure was simplified by the utilization of an

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Extrelut column and the required sample size was less than 100 μl. Using the proposed method, the serum levels of both PA and NAPA were determined in healthy volunteers and in patients with ventricular arrhythmias who were administered PA tablets orally.

EXPERIMENTAL

Apparatus and chromatographic conditions

A Hewlett-Packard Model 5710A gas chromatograph equipped with a nitrogen–phosphorus detector and a Model 3390A reporting integrator were used. The glass column (2.4 m × 2 mm I.D.) was packed with 3% silicone OV-17 on 80–100 mesh Chromosorb W. The carrier gas was high-purity nitrogen at a flow-rate of 30 ml/min. The detector purge was hydrogen and air at flow-rates of 3.5 and 70 ml/min, respectively. The injector port and detector temperatures were 300°C and the column temperature was programmed with an initial hold at 225°C for 8 min followed by an immediate increase to the final temperature of 260°C at 32°C/min. The detector sensitivity was adjusted to an offset of 30% (attenuation 2^6).

Reagents

HPLC-grade dichloromethane, and analytical-reagent-grade sodium hydroxide and methanol were obtained from Wako (Osaka, Japan). A pure standard of procainamide was kindly provided by Daiichi Seiyaku (Tokyo, Japan). N-Acetylprocainamide was obtained from Aldrich (Milwaukee, WI, U.S.A.). Propranolol, the internal standard, was kindly provided by ICI-Pharm (Osaka, Japan).

Analytical procedure

A 100-μl volume of serum was pipetted into a borosilicate glass culture tube (12 × 75 mm), then 1 ml of 2 *M* sodium hydroxide was added and mixed well. The samples were rendered alkaline and diluted in order to reduce the viscosity of serum. All of the solution was applied to an Extrelut column (E. Merck, Darmstadt, F.R.G.). After it had stood for 15 min at room temperature, the sample was eluted with 10 ml of dichloromethane and the eluate was collected, then 0.5 ml (500 ng) of a methanolic solution of propranolol as the internal standard (I.S.) was added. This one-step extraction was simple and the precision and recovery were excellent. Thus it was considered that the I.S. was needed only to prevent errors caused by imprecision in the amounts injected. All the eluate was evaporated to dryness, the residue was dissolved in 10 μl of methanol and 1 μl was injected into the GC system. Calibration graphs were prepared by adding PA and NAPA to blank human serum.

RESULTS AND DISCUSSION

Evaluation of the method

Fig. 1 shows the chromatograms of PA, NAPA and the I.S. PA and the I.S. were found at lower temperature and NAPA at higher temperature. With this pro-

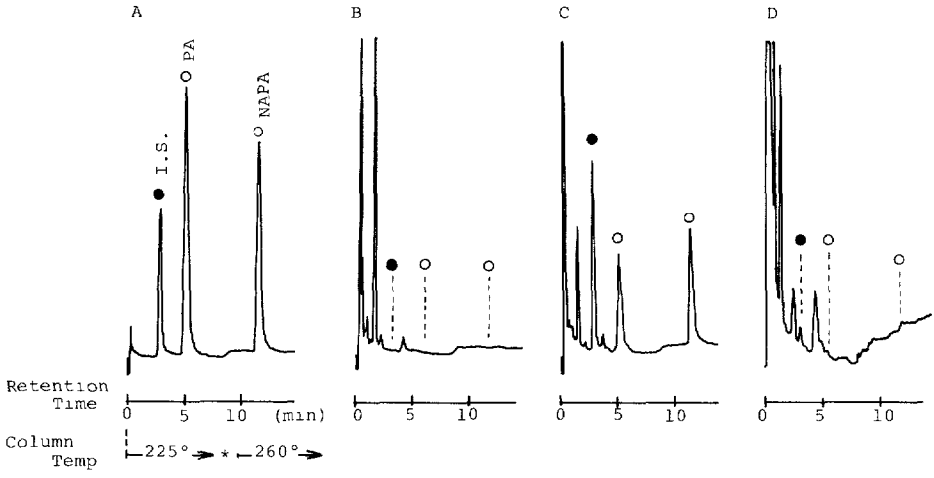

Fig. 1. Chromatograms of PA and NAPA. (A) Standard solution containing I.S., PA and NAPA; (B) blank serum from a normal adult; (C) patient's serum after oral administration of a 375-mg PA tablet; the PA and NAPA concentrations were found to be 2.5 and 3.0 μg/ml; (D) the same patient's serum treated as in (C), with FID. (*) Rate of temperature rise, 32 °C/min.

gramme, PA and NAPA can be detected simultaneously within a short time. Propranolol was the most suitable I.S. in view of the GC retention indices of cardiovascular drugs already reported [15].

The chromatogram of blank serum showed that no interfering materials were present. The same patient's serum that had been treated by the above extraction procedure was injected into the system with flame ionization detection (FID), and the results were compared with the chromatogram obtained with nitrogen–phosphorus detection (NPD). In contrast to FID, NPD gave relatively small peaks with blank serum and specific detection of PA and NAPA, and the baseline was more stable. The detection limits with on-column injection of PA and NAPA were 0.4 ng by NPD and 20 ng by FID. Using an Extrelut column for extraction, PA and NAPA were obtained quantitatively from serum without gel formation. As clean-up was also performed at the same time, the extraction process was simple and rapid. Most published methods include a liquid–liquid extraction procedure. However, it was considered that a solid–liquid extraction was more useful than liquid–liquid extraction for the above reason and also with respect to analysis time, recovery and precision.

Commonly used antiarrhythmic agents and other cardiovascular drugs were found not to interfere in this method.

The peak-area ratios of PA and NAPA to the I.S. were linearly related ($r = 0.9993$ and 0.9989, respectively) to concentration over the range 1–20 μg/ml in serum. The limit of quantitation of both PA and NAPA was 0.5 μg/ml. The recovery and precision were examined using human serum samples to which were added 1, 5 and 10 μg/ml PA and NAPA and EMIT control serum (6 μg/ml). The overall average recoveries of PA and NAPA were 98.6 and 98.3%, respectively.

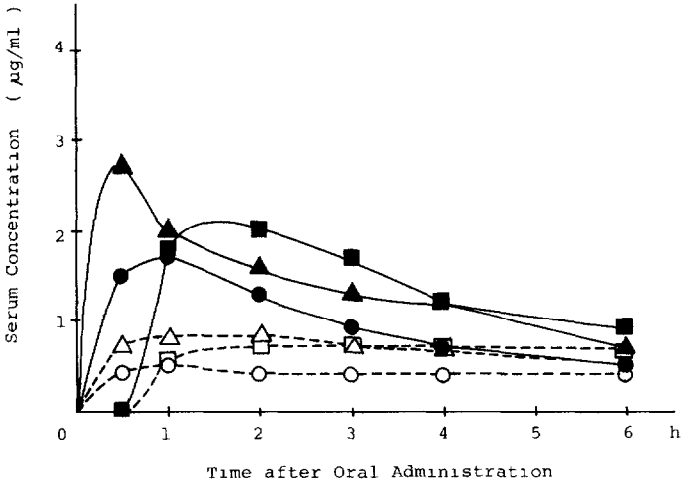

Fig 2. Serum levels of PA (closed symbols) and NAPA (open symbols) after a single oral administration of a PA tablet to normal adults. Pharmacokinetic parameters were calculated by the residual method in the open one-compartment model. Subject 1 (●), 42-year-old male, dose 250 mg per 48 kg body weight, $t_{1/2}=2.57$ h and $K_{el}=0.27$ h^{-1}; subject 2 (▲), 33-year-old male, dose 500 mg per 80 kg, $t_{1/2}=2.97$ h and $K_{el}=0.23$ h^{-1}; subject 3 (■), 28-year-old male, dose 500 mg per 66 kg, $t_{1/2}=2.85$ h and $K_{el}=0.242$ h^{-1}. Pharmacokinetic parameters were not calculated for NAPA.

The within-day precision was less than 3% ($n=10$) and the between-day reproducibility was less than 6% over ten days.

Analysis of PA and NAPA in human serum

The method was applied to a pharmacokinetic study of three healthy male volunteers. One of them was administered a 250-mg PA tablet orally and the other two a 500-mg tablet. Fig. 2 shows the serum concentrations of PA and NAPA after a single oral administration of a PA tablet. Pharmacokinetic parameters were calculated by the residual method in the open one-compartment model using these data and are given in Fig. 2. The elimination rate constant (K_{el}) and the biological half-life ($t_{1/2}$) of PA agreed with the values 0.3 h^{-1} and 2.42 h, respectively, given by Shimamura [16], indicating that the method is applicable to pharmacokinetic studies of PA. The concentration of NAPA was low because the volunteers had taken only a single administration of PA.

The method was also applied to monitoring the PA levels in some patients who had taken PA tablets and the values were compared with those determined using the EMIT system. The results are given in Table I. The concentrations of both PA and NAPA showed a good correlation ($r=0.992$ and 0.989, respectively) between the GC and EMIT methods; there was no significant difference between the two methods.

We conclude that GC with NPD is a selective and sensitive procedure for the simultaneous assay of PA and NAPA. It involves a simple extraction procedure and small sample size (less than 100 μl of serum). The described method is considered to be suitable for therapeutic monitoring of PA and NAPA.

TABLE I

THERAPEUTIC DRUG MONITORING

Patient	Duration	Dose per day (mg)	PA (μg/ml)		NAPA (μg/ml)		Concurrent medications
			GC	EMIT	GC	EMIT	
A	2 weeks	750	1.1	1.2	1.0	1.1	Alprenolol, nifedipine, trichlormethiazide
B	4 days	750	0.6 1.5	0.5 1.4	0.9 1.0	1.1 1.2	Spironolactone, digoxin, furosemide, dipyridamole
C	3 weeks	750	N.D.	0	N.D.	0	Nifedipine
D	2 weeks	750	1.0	0.8	0.9	0.9	
E	Long-term	1125	2.5	2.6	3.0	3.1	Nitrazepam
F	4 weeks	1000	0.6	0.5	1.5	1.3	

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